Naunyn-Schmiedeberg's Arch.Pharmacol. 281, 145-165 (1974).

Naunyn-Schmiedeberg's Arch. Pharmacol. 281, 145—165 (1974)

The Effects of 5-Hydroxytryptamine and Some Related Compounds on the Cat Superior Cervical Ganglion in situ

W. Haefely

Abt. für Experimentelle Medizin,
F. Hoffmann-La Roche & Co. AG, Basel, Schweiz

Received May 14, 1973

Summary. The effects of 5-hydroxytryptamine (5-HT) and related indole derivatives injected as i.a. bolus injections in a large dose range were studied in the cat superior cervical ganglion (SCG) by recording ganglionic and postganglionic electrical activity. 5-HT had two independent and opposite actions: a long-lasting inhibition of ganglionic transmission occurred already with extremely low doses and increased up to highest ones: both pre- and postsynaptic sites of action seem to be involved. In a medium and high dose range, the inhibitory effect was temporarily masked by a short-lasting stimulation and a depolarisation which was small when compared with that produced by nicotinic agents. The depolarization triggered a secondary hyperpolarization. The stimulant, but not the inhibitory action was very prone to tachyphylaxis.

LSD, methysergide, psilocybin and N,N-dimethyltryptamine (DMT) possess only the inhibitory property of 5-HT. The two N,N-dimethylated derivatives bufotenine and DMT, as well as the α-methylated 5-HT stimulated nicotinic receptors, bufotenine in addition to, and 5-hydroxy-α-methyltryptamine in the absence of typical 5-HT-like inhibitory and excitatory activity. Tryptamine had neither inhibitory nor excitatory 5-HT-like actions, it depolarized and blocked transmission by an unknown mechanism. 5-Hydroxy-α-methyltryptamine and α-methyltryptamine seem to have a marked affinity for, but no intrinsic activity at excitatory 5-HT receptors; they blocked the excitatory effect of 5-HT.

Key words: Sympathetic Ganglion — 5-Hydroxytryptamine — Indolealkylamines — 5-HT-Antagonists.

The stimulant action of 5-hydroxytryptamine (5-HT) on parasympathetic and sympathetic ganglion cells has been suggested soon after its identification and synthesis (Rocha e Silva *et al.*, 1953; Gaddum and Hameed, 1954; Trendelenburg, 1956a, b; Gaddum and Picarelli, 1957). In a few investigations, electrophysiological methods were used (Trendelenburg, 1957a; Reinert, 1960; Hertzler, 1961; Gyermek and Bindler, 1962a, b; de Groat and Volle, 1966; Jaramillo and Volle, 1968; Machová and Boška, 1969; Watson, 1970). The overall picture of the ganglionic effects of 5-HT which emerges from the literature is not very clear

and even controversial. Thus, dose-response relationships have not been worked out and the conditions under which 5-HT was studied differed quite markedly; not only stimulating, but also ganglionic inhibitory actions have been reported, although less often (Page and McCubbin, 1953; Reinert, 1960; Eccles and Libet, 1961; de Kalbermatten, 1962; Jécquier, 1965). In the brain, where 5-HT acts as a neurotransmitter, its microelectrophoretic application has revealed both excitatory and inhibitory actions (Tebēcis and di Maria, 1972).

It seemed desirable to study the effects of 5-HT in more detail in the cat superior cervical ganglion (SCG), the most frequently used preparation in ganglionic pharmacology. The ganglionic surface potential of the SCG as well as electrical events in its postganglionic nerves were recorded continuously. A number of agents chemically related to 5-HT were included in the study. Some preliminary results have been reported (Haefely, 1971).

Methods

The results were collected from experiments on 78 adult cats anaesthetized with urethane (700 mg/kg) and α-chloralose (60 mg/kg) i.p. and artificially ventilated. The details of the method are described in a preceding paper (Haefely, 1974a). Arterial blood pressure in a femoral artery, heart rate and body temperature were continuously monitored. The left SCG was exposed with its postganglionic nerves; the arterial and venous circulation remained intact. The *ganglionic surface (demarcation) potential* between the body of the ganglion and the crushed severed end of the postganglionic external carotid nerve was picked up by Ag-AgCl-electrodes connected to the tissue with cotton threads and fed into a d.c. amplifier. The evoked monophasic *postganglionic mass action potentials* were recorded between a platinum electrode on the intact nerve and the electrode on the crushed end which served as the common inactive lead for both recordings. For preamplification an a.c. amplifier was used. The ganglionic record was visualized on the lower beam, the postganglionic record on the upper beam of an oscilloscope and also stored on a tape recorder. Upward deflection of the lower trace indicates ganglionic depolarization (increased negativity of the surface of the ganglion). Photographs were made of the oscilloscope tracings either directly or after replay from the tape recorder using a polaroid camera. The preganglionic cervical sympathetic trunk was separated from the vagus and the aortic nerve, severed low in the neck and mounted on platinum stimulating electrodes. Rectangular monophasic impulses of 0.05 msec duration were supplied by a constant current stimulator. For submaximal stimulation the current was reduced to provide the desired amplitude of the postganglionic action potential, or the current intensity was held supramaximal, but the cervical sympathetic trunk was partially transected between the stimulating electrodes and the ganglion. All branches of the carotid artery except those supplying the ganglion were tied. A fine needle connected through a polyethylene tubing to a microsyringe (dead space of the system 0.05 ml) was inserted into the common carotid artery. Drugs were dissolved in saline and given as bolus injections (volume 0.1 ml).

All observations to be reported were made in at least 5 experiments unless stated otherwise.

The compounds used were: bufotenine (5-hydroxy-N,N-dimethyltryptamine) hydrogen oxalate, dihydroergotamine methansulphonate; 1,1-dimethyl-4-phenylpiperazinium (DMPP) iodide; N,N-dimethyltryptamine (DMT) hydrochloride; hexamethonium chloride; 5-hydroxytryptamine (5-HT) creatinine sulphate; 5-hydroxy-α-methyltryptamine (α-M-5-HT) creatinine sulphate; lysergic acid diethylamide (LSD) tartrate; methiothepin {1-[10,11-dihydro-8-(methylthio)dibenzo[b,f]thiepin-10-yl]-4-methylpiperazine}maleate; α-methyltryptamine hydrochloride; methysergide bimaleinate; morphine hydrochloride; ouabain; pilocarpine hydrochloride; psilocybin (3-[2-(dimethyl amino) ethyl]indol-4-ol dihydrogen phosphate ester); tryptamine sulphate.

Results

1. 5-Hydroxytryptamine (5-HT)

a) Stimulant Action. In all 51 ganglia tested, 5-HT in appropriate doses produced a ganglionic depolarization and evoked a brief asynchronous discharge in the postganglionic nerve (Fig. 1). The threshold dose varied between 3 and 10 nmoles (0.5 to 2 μg 5-HT base). Depolarization and firing never lasted longer than 5 sec. The amplitude of depolarization increased with the dose, but only up to 10 times the threshold dose (with DMPP, a gradual increase of depolarization was obtained over a dose range of 3 to 4 log units, Haefely, 1974a). With higher doses, depolarization did not further increase and was only immaterially prolonged. The maximal depolarization achieved with 5-HT amounted to only 10 to 15% of the maximal depolarization obtainable with nicotinic stimulants (Haefely, 1974b).

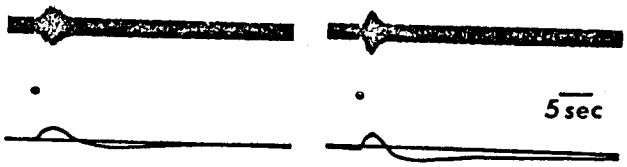


Fig. 1. Stimulant effect of 5-HT in the unconditioned, innervated cat SCG in situ. Response to 3 (left) and 10 nmoles i.a. (right) 5-HT. Background noise in the postganglionic record (above) is 10 μV. Ganglionic depolarization (below) after 10 nmoles is 0.2 mV

The sensitivity of the SCG to the stimulant action of 5-HT was enhanced by tetanic conditioning (50/sec for 20 to 30 sec); thereby, threshold doses were decreased by a factor of 3 to maximally 10. The size of maximal depolarization was not increased, but in fact decreased by prior tetanic stimulation. An even more dramatic sensitization was observed when 5-HT was given within 20 to 30 min after the injection of 10 nmoles isoprenaline (Fig. 2b), confirming the finding of de Groat

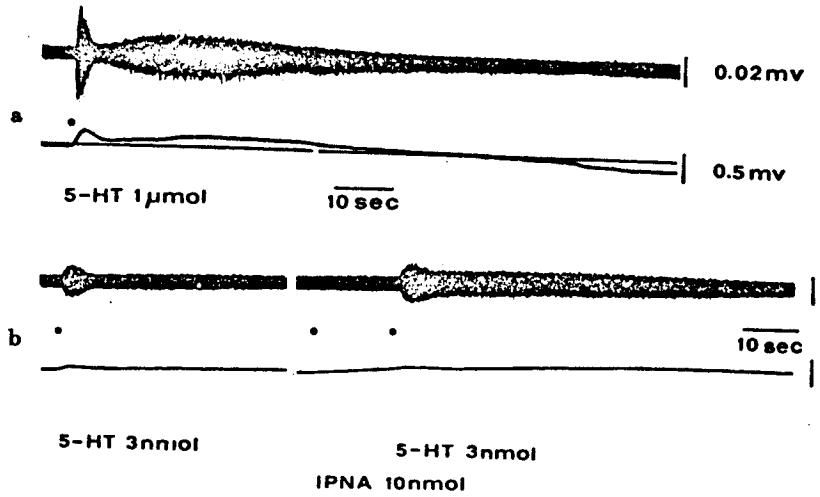


Fig. 2a and b. Bimodal depolarization and firing induced by 5-HT in the cat SCG in situ. a Chronically denervated ganglion. 5-HT (1 μmole) injected i.a. at the dot. b Normal ganglion. 5-HT (3 nmoles) before (left) and after (right) isoprenaline (IPNA 10 nmoles). Upper trace: postganglionic record (calibration 20 μV). Lower trace: ganglionic record (calibration 0.5 mV)

and Volle (1966). 6 chronically denervated ganglia did not significantly differ in their sensitivity from normal ganglia. High doses of 5-HT (above 30 nmoles) produced a bimodal pattern of depolarization and firing (Fig. 2a), as also seen in innervated ganglia after isoprenaline.

Ganglionic transmission was enhanced during ganglionic depolarization produced by doses of 5-HT not surpassing 10 times the threshold dose (Fig. 3). Facilitation occurred whether stimulation was submaximal or maximal; the percentual increase of the postganglionic spike height was of course larger when the stimuli were submaximal. The afternegativity of ganglionic action potentials was decreased, the amplitude of the afterpositivity, however, increased. Tachyphylaxis (desensitization) to the stimulant effect of 5-HT was very pronounced. After maximally depolarizing doses (10 to 30 nmoles) the effect of a subsequent test dose (10 nmoles) was blocked for up to 20 min. Desensitization was selective, since the stimulant effect of DMPP (Fig. 4) and pilocarpine or of KCl was not affected. Only after extremely high doses of 5-HT (1 μmole and more) was the stimulant effect of DMPP and KCl also reduced.

b) Depolarization and Depression of Transmission. Whereas threshold depolarizing doses and slightly higher ones briefly increased the

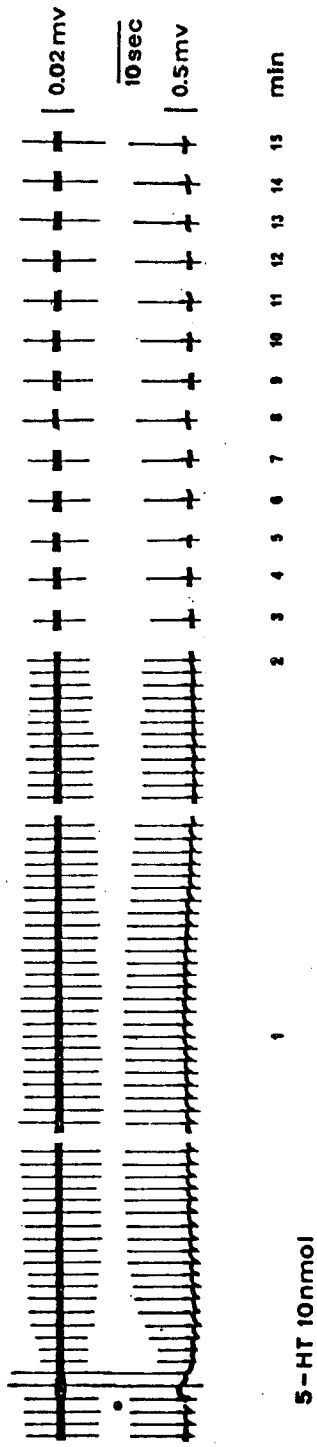


Fig. 3. Multiphasic effect of 5-HT (10 nmoles i.a.) on the surface potentials (lower trace) and postganglionic action potentials (upper trace) in the cat SCG in situ. Initial depolarization with facilitation, late hyperpolarization and inhibition interrupted by a delayed depolarization with transient recovery of transmission. Preganglionic stimulation at about 60% maximal strength and 0.5/sec

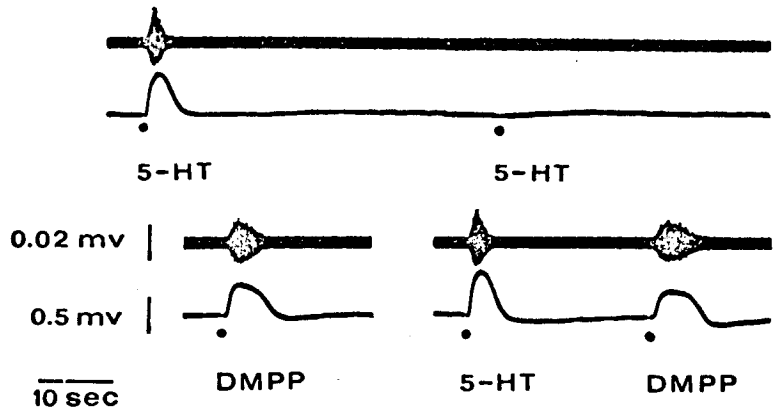


Fig. 4. Tachyphylaxis to the stimulant action of 5-HT. Chronically denervated cat SCG in situ. The effetc of 5-HT (10 nmoles) injected i.a. 50 sec after a first dose is completely abolished (upper row). The effect of DMPP injected 25 sec after the same dose of 5-HT remains unaltered (bottom row). Upper and lower traces are postganglionic and ganglionic records, respectively

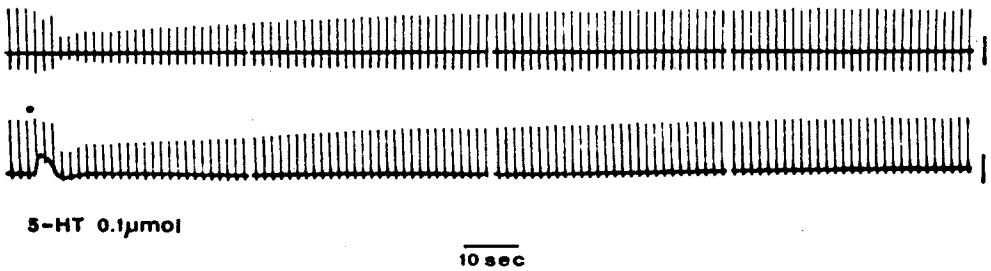


Fig. 5. Triphasic inhibition of ganglionic transmission in the cat SCG in situ after 5-HT 0.1 μmole i.a. (17.6 μg base). Depression is weak during the initial depolarization, marked during secondary hyperpolarization and moderate during a late faint hyperpolarization. Voltage scale 1 mV for both postganglionic (upper trace) and ganglionic (lower trace) records. Preganglionic maximal stimulation at 0.5/sec

amplitude of evoked postganglionic spikes, spike height was depressed during depolarization due to higher doses, although the peak of depolarization did not increase further (Fig. 5).

c) Secondary Phenomena Related to Depolarization. Depolarization produced by doses of 5-HT at least 3 times above threshold was immediately followed by a hyperpolarization which lasted twice to 5 times as long as the primary depolarization (Fig. 1). This early hyperpolarization was accompanied by a marked reduction of the amplitude of postganglionic spikes (Figs. 3 and 5) even when ganglionic transmis-

sion was enhanced during primary depolarization (Fig. 3). A reduction of the afterpositivity and an increase of the ganglionic afternegativity paralleled the early hyperpolarization, which was abolished by prior injection of ouabain in doses which markedly reduced posttetanic hyperpolarization and late hyperpolarization by DMPP (Haefely, 1974a).

d) Late Depression of Ganglionic Transmission. The late alterations following the injection of stimulating doses of 5-HT were very complex and more variable from one animal to another than the early modifications. Ganglionic transmission was variably depressed for a much longer period (up to 20 min) than lasted the secondary hyperpolarization described in the preceding paragraph. The late alterations were observed to proceed in one of two ways. Either the secondary hyperpolarization following the initial depolarization subsided or remained just visible and the postganglionic spike amplitudes recovered gradually over many minutes, as shown in Fig. 5. Alternatively, the secondary hyperpolization turned into a delayed depolarization of lower amplitude and longer duration than the initial one (Fig. 3). During this delayed depolarization, ganglionic transmission recovered partially or completely. After 1 or 2 min, the ganglionic demarcation potential reached the preinjection level or the ganglion was slightly hyperpolarized again. Ganglionic transmission was now depressed and recovered only slowly (Fig. 3).

e) Inhibitory Effect of Small Non-Stimulating Doses. Doses of 5-HT which were subthreshold with regard to stimulation of the ganglion were found to inhibit ganglionic transmission. This primary inhibition was usually accompanied by a weak hyperpolarization; however, even in those cases where an unambiguous hyperpolarization was not observed, the ganglionic afterpositivity was regularly decreased and the ganglionic afternegativity enhanced. The primary ganglionic inhibition was observed in 31 out of 38 ganglia studied. In the 7 ganglia in which substimulant doses of 5-HT did not depress postganglionic spike amplitude, control injections of saline or Krebs solution produced modifications of the ganglionic surface potential (usually a moderate depolarization), increased the ganglionic afterpositivity and affected the postganglionic spike amplitude in either direction. The smallest dose of 5-HT observed to produce a certain depression of postganglionic spikes was 1 picomole (5 ganglia) or approximately 0.2 ng of the base. However, the average threshold dose for primary inhibition was 30 to 100 picomoles, which is $^1/_{100}$ to $^1/_{300}$ of the threshold dose for stimulation in the corresponding ganglia. The primary inhibitory effect was more easily seen when preganglionic stimulation was submaximal. It was clearly dose-related except for doses approaching threshold for stimulation (Fig. 6). Obser-

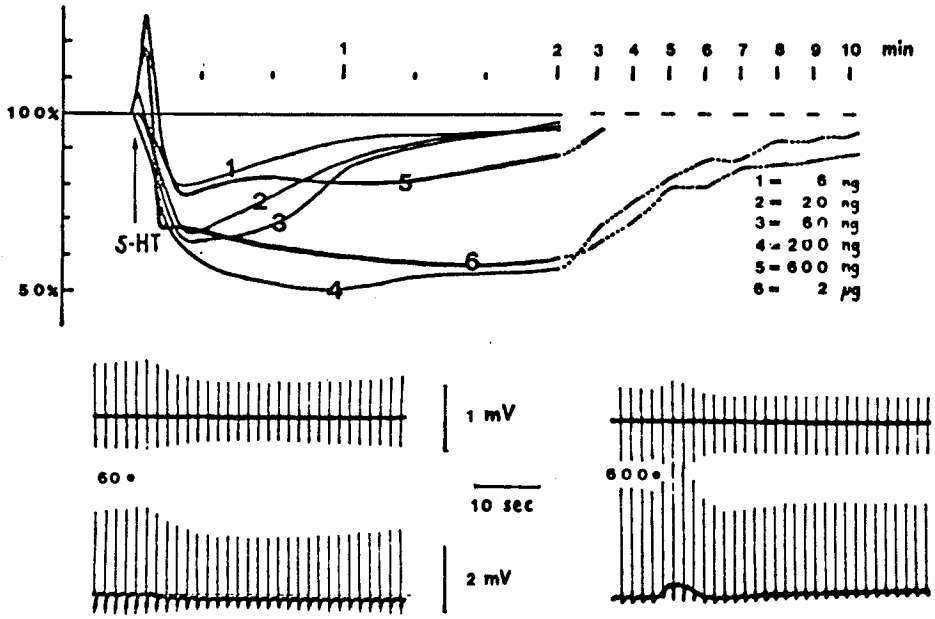


Fig. 6. Primary inhibitory effect of 5-HT in very low doses. Cat SCG in situ. 6 increasing doses were injected i.a. in the same animal at intervals of 20 min while stimulating the preganglionic nerve at about 70% maximal strength and 0.5/sec. The time course of the reduction of postganglionic spike amplitudes (upper trace) is shown graphically above. The figures on the curves indicate the doses of 5-HT base: *1* = 6 ng (30 picomoles); *2* = 20 ng (100 picomoles); *3* = 60 ng (0.3 nmole); *4* = 200 ng (1 nmole); *5* = 600 ng (3 nmoles); *6* = 2 µg (10 nmoles). Below two original records obtained with the indicated doses. The 3 highest doses produced an initial depolarization and facilitation of transmission

vation of the dose-effect relationship over a large dose range suggested that the primary inhibitory and the stimulant action of 5-HT were two independent phenomena. The inhibitory effect appeared with much smaller doses than stimulation did and it seemed to increase gradually over the whole dose range tested, although it was partially and temporarily masked when stimulating doses were injected. The rapid desensitization of the SCG to the stimulant action of 5-HT made it possible to verify this assumption. When a given stimulating dose or when increasing doses in the stimulant range were injected within short intervals, the ganglionic inhibitory effect was seen to add to that of the preceding dose.

f) Search for Antagonists of the Two Actions of 5-HT. The stimulant and inhibitory effects of 5-HT were not prevented by methysergide (up

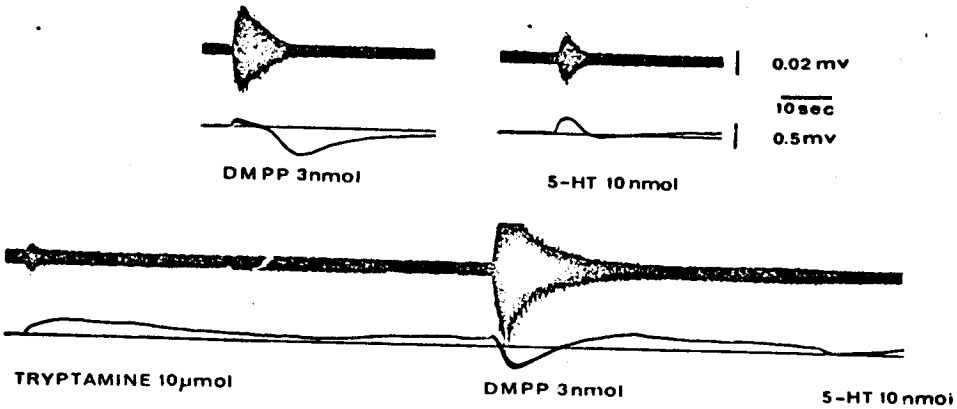


Fig. 7. Selective blockade of the stimulant effect of 5-HT (10 nmoles) and enhancement of the effect of DMPP (3 nmoles) after tryptamine (10 μmoles) in a chronically denervated cat SCG in situ. Control responses in the top row (upper and lower traces are postganglionic and ganglionic records). The bottom row is a continuous record

to 30 nmoles), LSD (up to 3 nmoles) and morphine (up to 10 nmoles) in doses which did not depress postganglionic action potentials in response to preganglionic stimuli. Also methiothepin, which was found to block inhibitory effects of 5-HT on single neurones in the lateral geniculate body (Tebēcis, 1972), was not an antagonist of either ganglionic effect of 5-HT. As will be described below, the stimulant action of 5-HT was prevented by α-methyl-5-hydroxytryptamine. 5-HT-induced primary inhibition, which greatly resembles that produced by α-adrenergic stimulants (de Groat and Volle, 1966; Haefely, 1969), was unaffected by dihydroergotamine in doses (0.1 to 0.3 μmoles) which blocked the inhibitory effect of adrenaline (10 nmoles).

2. Tryptamine

Tryptamine was investigated in 7 normal and 3 chronically denervated ganglia. It produced a dose-dependent ganglionic depolarization which was of much longer duration (up to 10 min) than that seen after 5-HT and which was never followed by a secondary hyperpolarization. Threshold doses were between 10 and 30 nmoles in both innervated and denervated ganglia. The maximum depolarization was somewhat larger than that after 5-HT. Tryptamine-induced depolarization was unaffected by hexamethonium. A postganglionic firing was never seen to accompany the depolarization in innervated ganglia; an extremely weak discharge occurred after the highest dose tested (10 μmoles) in two denervated

ganglia. Facilitation was never observed; on the contrary, transmission started to be depressed usually with threshold doses for depolarization. With the highest dose tested here (10 μmoles) ganglionic transmission was completely blocked. Whereas the stimulant effect of 5-HT given within a few minutes after tryptamine (1 μmole and more) was completely prevented, that of DMPP was markedly increased (Fig. 7).

3. α-Methyltryptamine

α-Methyltryptamine was studied in 1 normal and in 2 chronically denervated ganglia. It produced a weak depolarization in doses of 1 μmole and more without leading to any postganglionic discharges. In the normal ganglion, the smallest dose depressing ganglionic transmission was 1000 times higher than that of 5-HT. When given within 5 to 10 min after α-methyltryptamine, 5-HT produced no stimulation.

4. N,N-Dimethyltryptamine (DMT)

DMT, investigated in 8 normal ganglia and in 1 denervated ganglion, resembled 5-HT in producing a primary inhibition with hyperpolarization; it was approximately $^1/_{10}$ as potent as 5-HT. In doses of 0.1 μmole and more a long-lasting weak depolarization occurred, during which postganglionic discharges were seen and transmission was enhanced. Hexamethonium prevented depolarization and firing. The stimulant and facilitatory action of DMT was followed after several minutes by a long-lasting inhibition of ganglionic transmission, again similar to the response to 5-HT.

5. Bufotenine

This N,N-dimethyl derivative of 5-HT was investigated in 14 normal and 3 chronically denervated ganglia. In very small doses, bufotenine resembled 5-HT in producing a primary inhibition of ganglionic transmission, accompanied by a slight hyperpolarization or at least a reduction of the ganglionic afterpositivity and an increase of the afternegativity. It had about $^1/_{10}$ the potency of 5-HT. In doses around 10 nmoles a short-lasting depolarization with postganglionic firing and facilitation of transmission occurred, followed by long-lasting depression and signs of weak hyperpolarization. This effect, almost undistinguishable from that of 5-HT, was resistant to hexamethonium. With still higher doses, however, the similarity with 5-HT disappeared; the response to bufotenine now resembled more that to low doses of lobeline (Haefely, 1974b) in consisting of a moderate long-lasting depolarization which was accompanied by a postganglionic

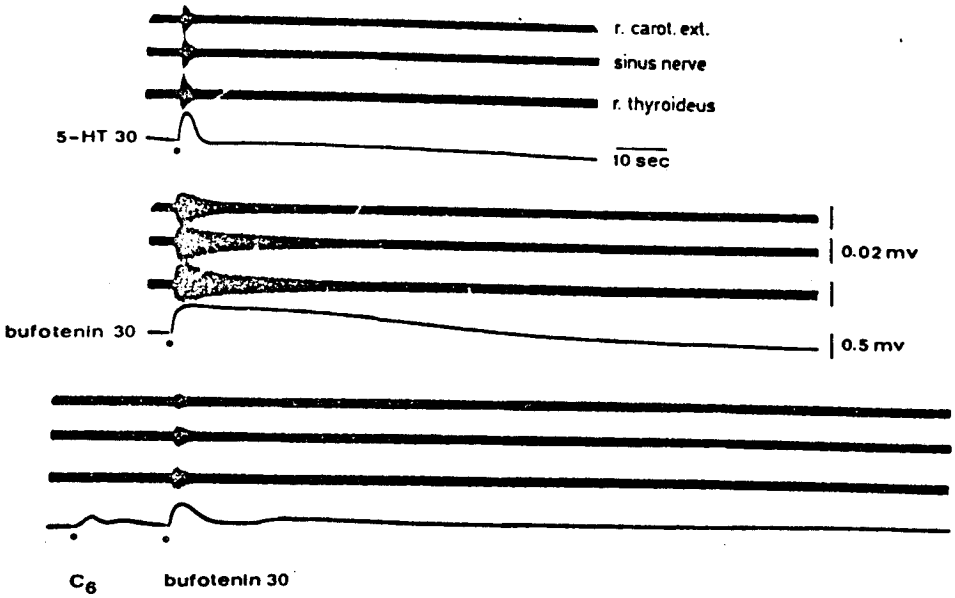


Fig. 8. Stimulant effect of identical doses of 5-HT and bufotenine (30 nmoles i.a.) in a chronically denervated cat SCG in situ. Recordings from the postganglionic external carotid nerve (top trace), from the carotid sinus nerve (second trace), the postganglionic thyroid branch (third trace), and from the ganglionic surface (bottom trace). After hexamethonium (10 μmoles), bufotenine produces only a brief 5-HT-like effect

discharge (Fig. 8). In higher doses bufotenine caused block of transmission. A dose of hexamethonium completely blocking ganglionic transmission profoundly altered the response to high doses of bufotenine: the long-lasting depolarization was converted into a stimulant effect like that elicited by 5-HT consisting of a short hexamethonium-resistant depolarization and firing. In Fig. 8 it can also be seen that there was a parallel stimulation of 5-HT- and nicotinic receptors in both the SCG and the sinus nerve endings.

6. 5-Hydroxy-α-methyltryptamine (α-M-5-HT)

Experiments on 10 normal and 2 chronically denervated ganglia revealed no 5-HT-like inhibitory and excitatory actions. α-M-5-HT produced a dose-dependent depolarization coinciding with facilitation of transmission at doses below 1 μmole and with ganglion block at

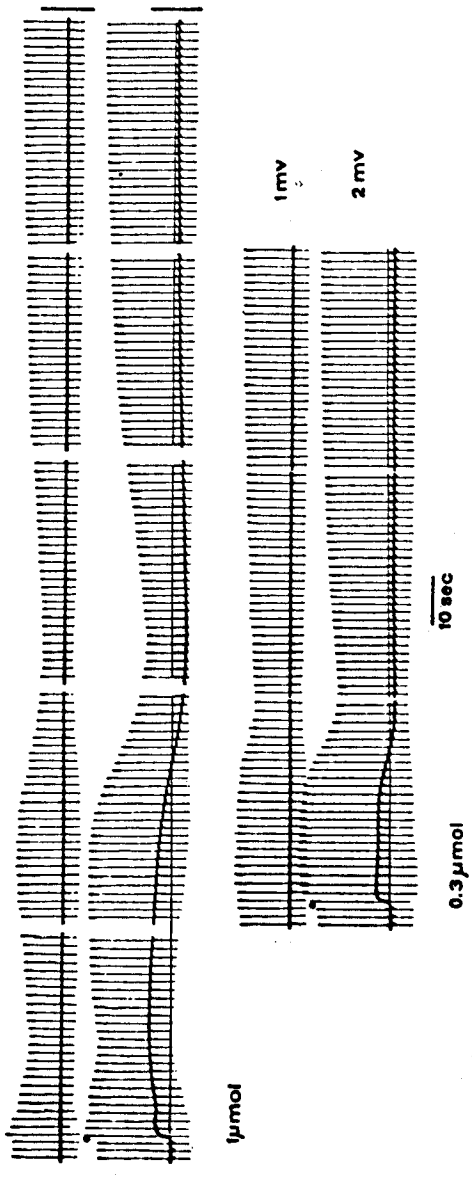


Fig. 9. Tetramethylammonium-like effects of two doses of 5-hydroxy-α-methyl-tryptamine, 1.0 µmole i.a. above and 0.3 µmole below in the cat SCG in situ. Maximal preganglionic stimulation at 0.5/sec. Upper and lower traces are the postganglionic (vertical calibration 1 mV) and ganglionic (vertical calibration 2 mV) records, respectively

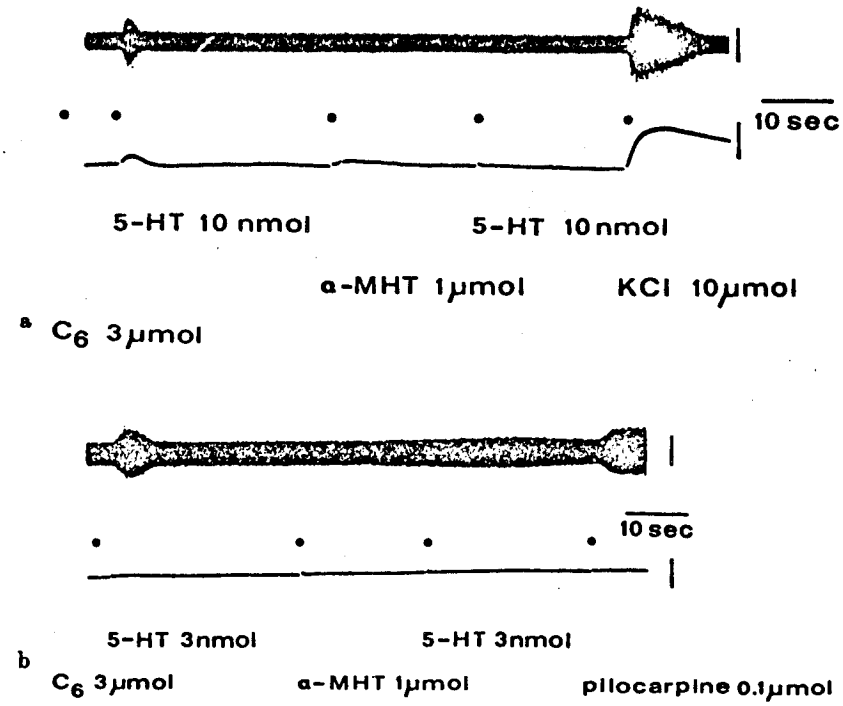
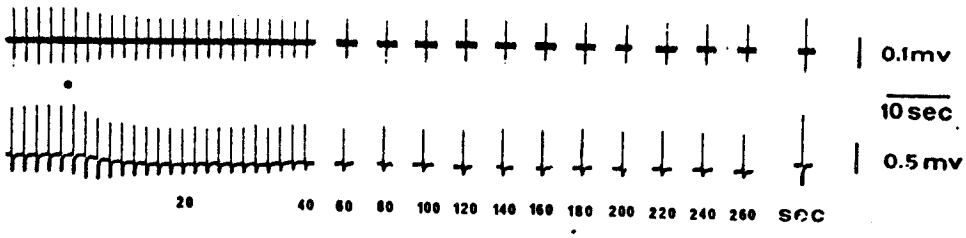


Fig. 10a and b. Selective abolition of the stimulant effect of 5-HT (10 nmoles) after 5-hydroxy-α-methyltryptamine (1 μmole) injected i.a. during blockade with hexamethonium in the cat SCG in situ (3 μmoles). The effects of KCl 10 μmoles (a) and of pilocarpine 0.1 μmole (b) were unaltered (control responses not shown). Voltage scale 20 μV for postganglionic record (upper trace) and 1.0 mV for ganglionic record (lower trace). Both records from the same cat

doses above 1 μmole. The depolarization was regularly followed by a late hyperpolarization and ganglionic depression (Fig. 9). All these effects were completely prevented by hexamethonium and, therefore, characterized as "nicotinic". In fact, the effect of α-M-5-HT strikingly resembled that of tetramethylammonium qualitatively (Haefely, 1974 b). There was, however, one effect of α-M-5-HT which was associated with 5-HT-receptors: when it was injected after blockade by hexamethonium, α-M-5-HT, although producing neither depolarization nor firing, abolished the stimulant effect of 5-HT for at least 10 min (Fig. 10). The blockade of 5-HT-receptors was selective, since neither KCl nor pilocarpine lost their effect. Whether the blockade of 5-HT-receptors by α-M-5-HT was surmountable or not, was not investigated.



LSD 10 nmol

Fig. 11. Ganglionic inhibitory effect of LSD (10 nmoles i.a.) in the cat SCG in situ. Preganglionic stimulation at 60% maximal strength and 0.5/sec. Numbers below the record indicate time in sec after the injection of LSD. Postganglionic record above, ganglionic record below

7. LSD

LSD was studied in 24 ganglia. In a purely descriptive way LSD can be characterized as acting like 5-HT but lacking any ganglionic stimulant properties. The only effect of LSD observed in the cat SCG was inhibition. Threshold doses were 10 to 30 times higher than those of 5-HT, i.e. 0.3 to 1 nmole (0.1 to 0.3 ng). The inhibition was accompanied by a slight hyperpolarization in the majority of ganglia, as well as by a decrease of the ganglionic afterpositivity and an increase of the afternegativity (Fig. 11). The depression of ganglionic transmission increased dose-dependently, whereas the hyperpolarization never reached the levels observed during the late hyperpolarization after DMPP (Haefely, 1974a) or the hyperpolarization induced by p-chloromercuribenzoate (Haefely, 1969). After 1 μmole of LSD, ganglionic transmission was completely blocked. The stimulant effect of DMPP was greatly reduced after such high doses. In one of two chronically denervated ganglia, LSD produced a weak hyperpolarization. The stimulant effect of 5-HT was decreased by LSD only in doses (100 nmoles and more) which produced a very marked depression of ganglionic transmission.

8. Psilocybin

In 11 ganglia, the effect of psilocybin was found to be almost identical with that of LSD, consisting of a depression of ganglionic transmission usually coincident, but not strictly correlated with hyperpolarization, decrease and increase of ganglionic afterpositivity and afternegativity, respectively. 5-HT was only 3 to 10 times more potent then psilocybin. No stimulant effect was ever observed. After high doses (around 0.1 μmole), a depression of the stimulant effect of 5-HT occurred in the absence of modifications of KCl- or DMPP-induced stimulation.

9. *Methysergide*

Methysergide was studied in only two cats. It produced depression of ganglionic transmission only, and, therefore, may best be compared with LSD, which was, however, 10 to 30 times more potent. In strongly depressant doses (above 0.3 μmole) methysergide also reduced the stimulant effect of 5-HT.

Discussion

The present study has revealed a very complex action of 5-HT in the sympathetic SCG of the cat. In addition to its already known stimulant effect an inhibitory property of 5-HT was found.

The *ganglionic stimulant action of 5-HT* differs markedly from that of nicotinic and muscarinic agents as well as of polypeptides (Haefely, 1970) and histamine. 5-HT produces a very brief depolarization during which postganglionic discharges are recorded. In unconditioned ganglia and under the experimental conditions used here the threshold dose was approximately 5 nmoles (1 μg of the base). Depolarization increased in a dose-dependent manner only in a very narrow range. With 10 times the threshold dose, the maximal depolarization was reached; this depolarization amounted to not more than 10 to 15% of that obtained by maximally effective doses of nicotinic agents (Haefely, 1974b). The reason for this is not clear and the answer could be obtained only by intracellular recording from single cells. Only a small proportion of the cells in the ganglion may possess the receptors responsible for 5-HT-induced depolarization—which could be as complete as that produced by nicotinic agents—and the recording from the whole mass of ganglionic tissue results in only a small ganglionic negativity. Alternatively, all cells might have 5-HT-receptors, but the induced membrane alteration does not permit more than a depolarization just around threshold for the firing of an action potential. The maximal amplitude of the asynchronous postganglionic firing after 5-HT also was always smaller than that observed after nicotinic agents or KCl; this is consistent with both alternatives. The electrogenesis of 5-HT-induced depolarization is unknown; however, in contrast to Jaramillo and Volle (1968) but in accordance with Machová and Boška (1969) a secondary hyperpolarization was observed to follow the initial depolarization. This may be taken as indirect evidence for 5-HT-induced accumulation of intracellular Na^+, since the secondary hyperpolarization in all respects resembled that observed after nicotinic agents and attributed to the activation of an electrogenic Na^+-pump (see Haefely, 1974a). The very short duration of depolarization is probably due to receptor desensitization. In fact, a very striking and

regular finding was the refractory state of the ganglion to stimulation by 5-HT for up to 10 min following a preceding dose of 5-HT. Receptor desensitization or tachyphylaxis seems to be characteristic property of 5-HT both for smooth muscle stimulation (Gaddum, 1953) and for excitatory action on brain neurones (Tebēcis, personal communication).

Ganglionic transmission was facilitated during the depolarization produced by medium doses of 5-HT. Facilitation never outlasted the depolarization; it is, therefore, surprising that by recording nictitating membrane contractions in response to preganglionic stimulation, the facilitating effect of 5-HT was observed to last for a considerably longer time (Trendelenburg, 1956b). It is suggested that in this experimental situation the very protracted response of the smooth muscle to the evoked asynchronous action potentials simulated the occurrence of an increased number of action potentials in response to single preganglionic impulses. The increased tone of the nictitating membrane during the observed facilitation is in keeping with this view.

In confirmation of earlier reports (de Groat and Volle, 1966), isoprenaline enhanced the stimulant effect of 5-HT and enhanced a low-amplitude firing during the delayed depolarization occurring after high doses. Orthodromic tetanic conditioning also facilitated the stimulation of the ganglion by 5-HT, but less markedly than did isoprenaline.

After high doses of 5-HT depolarization coincided with depression of ganglionic transmission. In view of the low amplitude of depolarization—as compared with that elicited by nicotine and DMPP—one hesitates to explain the inhibition by the depolarization and to call it "depolarization block".

The stimulant effect of 5-HT was not antagonized by LSD in doses which left the response to DMPP unaltered, as already found by Gyermek and Bindler (1962a) in the cat mesenteric ganglion. Morphine has been reported to block 5-HT-induced postganglionic activity (Trendelenburg, 1957b; Gyermek and Bindler, 1962a). In the present investigation, morphine depressed ganglionic transmission in doses above 10 nmoles (3 μg) i.a.; however, 30 to 100 times higher doses were necessary for antagonizing stimulation by 5-HT; these doses also depressed the effect of DMPP. At least in the cat SCG, morphine cannot be considered to be a selective antagonist of 5-HT. However, the stimulant effect of 5-HT was selectively blocked by 5-HT itself (probably by receptor desensitization), by 5-hydroxy-α-methyltryptamine, by tryptamine, and to lesser degree by bufotenine and psilocybin. The value of these compounds as 5-HT-antagonists is of course greatly reduced by their other actions on the ganglion.

Ganglionic Depressant Effects of 5-HT. Depression of ganglionic transmission occurs to a varying degree at various times after an injection of 5-HT and greatly depends on the dose.

The depression during depolarization produced by high doses has already been mentioned.

Inhibition is most pronounced during the brief hyperpolarization which immediately follows the initial depolarization by medium and high doses. This phase resembles the late hyperpolarization coinciding with ganglionic inhibition after small doses of acetylcholine in the presence of hyoscine (Haefely, 1974b), and it is reasonable to assume that both are due to the activity of an electrogenic Na^+-pump.

The reactive hyperpolarization lasts only slightly longer than the initial depolarization by which it is triggered. The response to 5-HT then follows either of two courses. Either the ganglionic polarization remains at a slightly positive level and gradually returns to the base line in parallel with a recovery of the reduced postganglionic action potentials; this recovery may last up to 20 min. Alternatively the early phase of reactive hyperpolarization and marked depression of ganglionic transmission is followed by a second phase of depolarization which has a smaller amplitude and a longer duration than the initial phase of depolarization; during this late depolarization ganglionic transmission partially or fully recovers. The amplitude of the postganglionic action potentials then decreases again and a slight hyperpolarization occurs; recovery of both changes usually takes about 20 min. The delayed depolarization just mentioned has been described by Machová and Boška (1969) and increases with increasing doses. No explanation can be offered; it seems unlikely that the delayed depolarization is due to the stimulation of the receptors mediating the initial depolarization e.g. by recirculating 5-HT, because receptor desensitization should prevent it. That it represents a true depolarization of ganglion cells is suggested by the asynchronous firing which accompanies this late ganglionic negativity in the denervated ganglion and in the innervated ganglion treated with isoprenaline. The late and longlasting inhibition of ganglionic transmission, which was always parallelled by a reduction of the ganglionic afterpositivity, seemed to be due do an action of 5-HT completely unrelated to its stimulant property.

The observations of a pure inhibitory effect of 5-HT in low, non-stimulant doses adds weight to this interpretation. In the majority of ganglia inhibition was the only effect seen with doses down to at least $^1/_{100}$ the threshold dose for stimulation. In this substimulant dose-range the ganglionic depression increased with the dose and it seems reasonable to assume that this dose-related inhibition further increased

with stimulant doses, the real time course and intensity now becoming temporarily masked by the excitatory effect of the amine. Support for this interpretation was obtained by results obtained when increasing doses of 5-HT were given at short intervals. Due to the fact that "tachyphylaxis" occurred to the stimulant but not to the inhibitory effect, an increasing cumulative depression was observed. In most cases the 5-HT-induced inhibition of ganglionic transmission coincided with a small hyperpolarization; experience with many kinds of hyperpolarization (afterpositivity of an action potential, secondary hyperpolarization after nicotinic stimulation, hyperpolarization by p-chloromercuribenzoate), however, made it appear unlikely that the 5-HT-induced depression was fully accounted for by hyperpolarization. A depressant effect on presynaptic transmitter release seems to be at least partially involved, since extremely high doses of 5-HT (1 μmole and more) had to be given before a reduction of DMPP-induced depolarization occurred. Such a mixed pre- and postsynaptic site of action has also been postulated by Tebēcis and di Maria (1972) for the inhibitory effect of 5-HT on geniculo-cortical relay neurones. Methiothepin, which blocked this inhibitory effect (Tebēcis, 1972) and that of 5-hydroxytryptophan on ponto-geniculo-occipital waves (Monachon *et al.*, 1972), did not prevent the 5-HT depression in the ganglion in doses which had no inhibitory effect themselves. This was disappointing, because it was hoped to find in the cat SCG a useful model for screening possible central 5-HT-antagonists. It seems that the receptors mediating inhibition in the brain differ from those mediating depression of ganglionic transmission. Interestingly, an inhibitory effect of 5-HT was not observed in the cat ciliary ganglion (Schaffner, 1973), whereas the parasympathetic ganglia of the cat urinary bladder possess both inhibitory and excitatory receptors for 5-HT (de Groat and Saum, 1972).

The ganglionic actions of the other indole derivatives can be briefly described in comparison with those of 5-HT.

Tryptamine produced a hexamethonium-resistant long-lasting monophasic depolarization virtually without eliciting postganglionic discharges. Transmission was never enhanced but strongly depressed. During several minutes following the injection of tryptamine, the stimulant effect of DMPP was increased, that of 5-HT prevented. It is highly unlikely that in tryptamine-induced depolarization those receptors were involved which mediate 5-HT-induced depolarization and firing. The use of the term "tryptamine receptors" (Gaddum, 1953) for those involved in the 5-HT effects is inappropriate at least for the cat SCG. If anything, tryptamine acts as an antagonist of the excitatory 5-HT receptors.

α-Methyltryptamine seemed to differ from tryptamine only by its lower potency.

N,N-Dimethyltryptamine (DMT) shared with 5-HT the inhibitory action on ganglionic transmission, being about $^1/_{10}$ as potent. In rather high doses it produced a long-lasting nicotinic stimulation.

Bufotenine was only slightly less potent than 5-HT in depressing ganglionic transmission in substimulant doses. Like 5-HT, and in similar doses, bufotenine produced a brief depolarization and firing, followed by hyperpolarization and depression. In still higher doses, a long-lasting depolarization occurred which can best be compared with that observed with lobeline (Haefely, 1974b). Hexamethonium converted this lobeline-like effect into one indistinguishable from that of 5-HT. Bufotenine in high doses, therefore, combined all the effects of 5-HT with those of the nicotine-like lobeline (Haefely, 1974b). Tachyphylaxis to the of 5-HT stimulant action also occurred with bufotenine. Similar findings were made by Gyermek and Bindler (1962b) in the cat mesenteric ganglion.

LSD is best described as a compound having the inhibitory actions of 5-HT only; being $^1/_{10}$ to $^1/_{30}$ as potent than 5-HT, it behaved like 5-HT after desensitization of the excitatory receptors. Only in very high doses, markedly depressing ganglionic transmission, did LSD block the stimulant effect of 5-HT; that of DMPP was, however, equally depressed.

The effect of *psilocybin* were very similar to those of LSD. Psilocybin shared with 5-HT only the ganglionic depressant action, being 3 to 10 times less potent. After high doses of psilocybin, the stimulant effect of 5-HT was depressed while that of DMPP and KCl remained unaltered; this points to some affinity for excitatory 5-HT receptors and a presynaptic site of action for depression of the ganglionic transmission. The lack of 5-HT-like stimulation is in agreement with Gyermek and Bindler (1962b), who did not find, however, the quite marked inhibitory action of psilocybin.

In the present experiments *methysergide* produced the same effects as LSD; 10 to 30 times higher doses were required with methysergide. An inhibition was also the only effect of methysergide found by de Kalbermatten (1962) in the isolated rat SCG.

In conclusion, the cat SCG contains at least two types of receptors for 5-HT, one type mediating depression of ganglionic transmission probably by a combination of pre- and postsynaptic effects, the other type mediating depolarization and discharges of ganglion cells. Much lower doses of 5-HT are required for inhibition than for excitation. A very marked desensitization of the excitatory but not of the inhibitory receptors is a characteristic property of 5-HT. The

electrogenesis of 5-HT-induced depolarization is not yet known, but evidence was obtained that it might not differ too much from the nicotine-induced membrane conductance increase. The response of the ganglion to high doses of 5-HT is complicated by the simultaneous action on inhibitory and excitatory receptors and by secondary phenomena triggered by the depolarization. Morphine was devoid of a selective 5-HT-antagonistic property.

Acknowledgment. The competent technical assistance of Miss U. Holz with the experiments and of Mr. H. Wolfgang with the electronic equipment are gratefully acknowledged. I thank Drs. G. Häusler and A. Hürlimann for critical reading of the manuscript.

References

De Groat, W. C., Volle, R. L.: Interaction between the catecholamines and ganglionic stimulating agents in sympathetic ganglia. J. Pharmacol. exp. Ther. 154, 200–215 (1966)

De Groat, W. C., Saum, W. R.: Sympathetic inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. J. Physiol. (Lond.) 220, 297–314 (1972)

Eccles, R. M., Libet, B.: Origin and blockade of the synaptic responses of curarized sympathetic ganglia. J. Physiol. (Lond.) 157, 484–503 (1961)

Gaddum, J. H.: Tryptamine receptors. J. Physiol. (Lond.) 119, 363–368 (1953)

Gaddum, J. H., Hameed, K. A.: Drugs which antagonize 5-hydroxytryptamine. Brit. J. Pharmacol. 9, 240–248 (1954)

Gaddum, J. H., Picarelli, Z. P.: Two kinds of tryptamine receptor. Brit. J. Pharmacol. 12, 323–328 (1957)

Gyermek, L., Bindler, E.: Blockade of the ganglionic stimulant action of 5-hydroxytryptamine. J. Pharmacol. exp. Ther. 135, 344–348 (1962a)

Gyrmek, L., Bindler, E.: Action of indole alkylamines and amidines on the inferior mesenteric ganglion of the cat. J. Pharmacol. exp. Ther. 138, 159–164 (1962b)

Haefely, W.: Effects of catecholamines in the cat superior cervical ganglion and their postulated rôle as physiological modulators of ganglionic transmission. Progr. Brain Res. 31, 61–72 (1969)

Haefely, W.: Some actions of bradykinin and related peptides on autonomic ganglion cells. In: Bradykinin and related kinins, pp. 591–599. Plenum Press 1970

Haefely, W.: Effects of serotonin (5-HT) and some related indole compounds in a mammalian sympathetic ganglion. Experientia (Basel) 27, 112 (1971)

Haefely, S.: The effects of 1,1-dimethyl-4-phenyl-piperazinium (DMPP) in the cat superior cervical ganglion in situ. Naunyn-Schmiedeberg's Arch. Pharmacol. 281, 57–91 (1974a)

Haefely, W.: The effects of various "nicotine-like" agents in the cat superior cervical ganglion in situ. Naunyn-Schmiedeberg's Arch. Pharmacol. 281, 93–117 (1974b)

Hertzler, E. C.: 5-Hydroxytryptamine and transmission in sympathetic ganglia. Brit. J. Pharmacol. 17, 406–413 (1961)

Jaramillo, J., Volle, R. L.: A comparison of the ganglionic stimulating and blocking properties of some nicotinic drugs. Arch. int. Pharmacodyn. 174, 88–97 (1968)

Jéquier, E.: Effet de la sérotonine sur la transmission synaptique dans le ganglion sympathique cervical isolé du rat. Helv. physiol. pharmacol. Acta 23, 163–179 (1965)

Kalbermatten, J. P. de: Effet de la sérotonine sur l'hypersensibilité de dénervation du ganglion sympathique cervical isolé du rat. Helv. physiol. pharmacol. Acta 20, 293–315 (1962)

Machová, J., Boška, D.: The effect of 5-hydroxytryptamine, dimethylphenylpiperazinium and acetylcholine on transmission and surface potential in the cat sympathetic ganglion. Europ. J. Pharmacol. 7, 152–158 (1969)

Manachon, M. A., Burkard, W. P., Jalfre, M., Haefely, W.: Blockade of central 5-hydroxytryptamine receptors by methiothepin. Naunyn-Schmiedeberg's Arch. Pharmacol. 274, 192–197 (1972)

Page, I. H., McCubbin, J. W.: The variable arterial pressure response to serotonin in laboratory animals and man. Circulat. Res. 1, 354–362 (1953)

Reinert, H.: The depolarizing and blocking action of amphetamine in the cat's superior cervical ganglion. In: Vane, Wolstenholen and O'Conner (eds.): Adrenergic mechanisms. CIBA Foundation symposium, pp. 373–379. London: Churchill Ltd. 1960

Rocha e Silva, M., Valler, I. R., Picarelli, Z. P.: A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. Brit. J. Pharmacol. 8, 378–388 (1953)

Schaffner, R.: Morphologie, Physiologie und Pharmakologie des Ganglion ciliare der Katze. Ph. D. Thesis, University of Basel (1973)

Tebēcis, A. K.: Antagonism of 5-hydroxytryptamine by methiothepin shown in microelectrophoretic studies of neurones in the lateral geniculate nucleus. Nature (Lond.) 238, 63–64 (1972)

Tebēcis, A. K., di Maria, A.: A re-evaluation of the mode of action of 5-hydroxytryptamine on lateral geniculate neurones: comparison with catecholamines and LSD. Exp. Brain Res. 14, 480–493 (1972)

Trendelenburg, U.: The actions of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. Brit. J. Pharmacol. 11, 74–80 (1956a)

Trendelenburg, U.: Modification of transmission through the superior cervical ganglion of the cat. J. Physiol. (Lond.) 132, 529–541 (1956b)

Trendelenburg, U.: The action of histamine, pilocarpine and 5-hydroxytryptamine on transmission through the superior cervical ganglion. J. Physiol. (Lond.) 135, 66–72 (1957a)

Trendelenburg, U.: The action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. Brit. J. Pharmacol. 12, 79–85 (1957b)

Watson, P. J.: Drug receptor sites in the isolated superior cervical ganglion of the rat. Europ. J. Pharmacol. 12, 183–193 (1970)

W. Haefely
Abt. f. Experimentelle Medizin
F. Hoffmann-La Roche & Co. AG
CH-4002 Basel/Schweiz